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The role of clusterin (CLU) in extracellular protein folding quality control

Amy Ruth Wyatt
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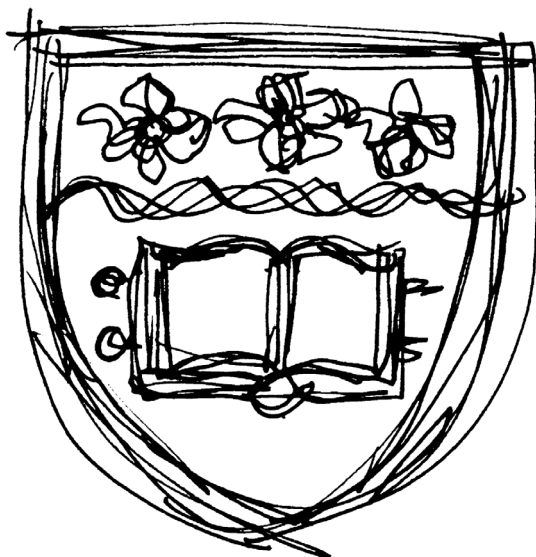
The Role of Clusterin (CLU) in Extracellular Protein Folding Quality Control

By

Amy Ruth Wyatt

Bachelor of Biotechnology (Advanced) Honours 1

This thesis is presented as part of the requirements for the degree of
Doctor of Philosophy



School of Biological Sciences
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September 2009

DECLARATION OF AUTHENTICITY

This thesis is submitted in accordance with the regulations of the University of Wollongong in fulfilment of the Degree of Doctor of Philosophy. It does not include any material published by another person except where due reference is made in the text. The experimental work described in this thesis is original and has not been submitted for a degree to any other university.

Amy Ruth Wyatt

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ABSTRACT

Processes to attain and maintain the correct three-dimensional shape, known as the native conformation, of proteins are vital. However, certain conditions including thermal and oxidative stress may cause proteins to partially unfold and aggregate. Intracellular and/or extracellular protein aggregates have been identified in a large number of diseases, including Alzheimer's disease, arthritis and type II diabetes. While intracellular quality control for the folding state of proteins is well characterized, corresponding mechanisms for extracellular protein folding quality control have yet to be described.

Clusterin (CLU) is an abundant extracellular chaperone that can stabilize proteins and prevent their precipitation during exposure to elevated temperatures or oxidative stress *in vitro*. The work described here demonstrates that CLU stabilizes stressed client proteins by forming soluble, high molecular weight (HMW) complexes with them. The maximum loading of CLU appears to be at a mass ratio of CLU:stressed client protein of approximately 1:2 (irrespective of the identity of the client protein or the temperature used to induce heat stress). It was demonstrated that various human plasma proteins show increased association with CLU after plasma is subjected to mild shear stress or oxidative stress at 37°C - the most abundant of these was fibrinogen (FGN) which co-purified with CLU from stressed plasma. *In vitro*, using purified proteins, heat stress of 45°C for 12 h was required to induce FGN precipitation and the formation of HMW CLU-FGN complexes. Size exclusion chromatography (SEC) of the stressed plasma suggested that a portion of the complexes formed in plasma between CLU and FGN may be similar in mass to those formed *in vitro*.

Using surface plasmon resonance, although CLU was found to bind to megalin, only minimal (or no) binding of HMW complexes formed between CLU and glutathione-S-transferase, citrate synthase or lysozyme was detected. Similarly, negligible binding of these complexes to low density lipoprotein receptor superfamily members expressed on the surface of the rat yolk sac cell line BN was detected. However, the complexes were shown to preferentially bind to the surface of BN cells, peripheral human monocytes and rat hepatocytes (more so than uncomplexed CLU or client proteins). In all cases, this binding was inhibited by fucoidin (a scavenger receptor inhibitor). Confocal microscopy suggested that binding of HMW CLU-stressed protein complexes to the

surface of BN cells or rat hepatocytes was followed by their internalization into lysosomes. Furthermore, Western blotting showed that hepatocytes were able to degrade the HMW CLU-stressed protein complexes and that the degradation was almost completely abolished by inhibiting lysosomal proteases with chloroquine. The results of *in vivo* biodistribution studies in Sprague Dawley rats were highly consistent for several different HMW CLU-stressed protein complexes. Intravenous ¹²³I-labelled HMW CLU-stressed protein complexes were cleared more efficiently from circulation compared to free CLU and the uncomplexed client proteins. The liver and to a lesser degree the spleen appeared to be the key organs responsible for the uptake of complexes and this uptake was inhibited by pre-injection of the animals with fucoidin.

The findings of this study suggest an important role for CLU in global quality control of extracellular protein folding. It appears likely that stressed (partially unfolded) extracellular proteins are stabilized and held in solution by CLU and that CLU-stressed protein complexes are subsequently taken up by fucoidin-inhibitable cell surface receptors for subsequent degradation within lysosomes. The precise physical characteristic or binding site that targets CLU-stressed protein complexes for receptor-mediated uptake remains to be identified and further work is needed to determine the particular receptor(s) involved. The findings of this study support a model in which complexation with CLU is an important first step in the targeted disposal of stressed proteins via scavenger-like endocytic receptors.

TABLE OF CONTENTS

Declaration of authenticity.....	ii
ACKNOWLEDGEMENTS.....	iii
ABSTRACT.....	iv
TABLE OF CONTENTS.....	vi
LIST OF FIGURES.....	xiii
LIST OF TABLES.....	xx
ABBREVIATIONS.....	xxi
Publications and conference presentations.....	xxv
1 INTRODUCTION.....	1
1.1 Protein folding and unfolding.....	1
1.1.1 Protein folding.....	1
1.1.2 Protein unfolding.....	2
1.2 Protein aggregation and disease.....	5
1.2.1 Types of protein deposits.....	7
1.2.2 Cytotoxicity.....	8
1.3 Protein folding quality control.....	10
1.3.1 Intracellular protein folding quality control.....	11
1.3.1.1 The ubiquitin-proteasome system.....	12
1.3.1.2 Lysosomal degradation.....	14
1.3.1.3 Molecular chaperones.....	16
1.3.2 Extracellular protein protein folding quality control.....	18
1.3.2.1 Extracellular proteolytic systems.....	19
1.3.2.2 Normally intracellular chaperones found extracellularly.....	20
1.3.2.3 Extracellular chaperones.....	21
1.3.2.3.1 Clusterin (CLU).....	21
1.3.2.3.2 Haptoglobin (Hp).....	26
1.3.2.3.3 α_2 -Macroglobulin (α_2 M).....	27
1.3.2.3.4 Extracellular refolding chaperones?.....	28
1.4 A model for the disposal of stressed proteins.....	29
1.4.1 Potential receptors for the disposal of CLU-stressed protein complexes.....	30
1.4.1.1 The low density lipoprotein (LDL) receptor superfamily.....	30
1.4.1.2 Scavenger Receptors (SRs).....	33

1.4.1.2.1	Scavenger Receptor-Class A (SR-A)	35
1.4.1.2.2	Scavenger Receptor-Class B (SR-B)	36
1.4.1.2.3	Other Scavenger Receptors	37
1. 5	Objectives	38
2	GENERAL MATERIALS AND METHODS	39
2. 1	Materials	39
2. 2	CLU purification from human plasma	41
2. 3	Estimation of protein concentration	41
2. 4	SDS-PAGE	42
2. 5	Western blot	42
2. 6	Protein precipitation assays	43
2. 7	Preparation of residual stressed control proteins	43
2. 8	Size exclusion chromatography (SEC)	44
2. 9	ELISA to detect HMW complexes formed <i>in vitro</i>	44
2. 10	Biotinylation	45
2. 11	Mass spectrometry	45
2. 12	Circular dichroism (CD)	46
3	FORMATION OF CLU-STRESSED PROTEIN COMPLEXES AT PHYSIOLOGICALLY RELEVANT TEMPERATURES	47
3. 1	Introduction	47
3. 2	Materials and methods	48
3.2.1	Purification of recombinant GST	48
3.2.2	Protein precipitation assays	49
3.2.3	Preparation of residual stressed controls	49
3.2.4	SEC	49
3.2.5	ELISA to detect HMW CLU-stressed protein complexes formed <i>in vitro</i>	50
3.2.6	Native gel electrophoresis	50
3.2.7	Ion-exchange chromatography	50
3.2.8	CD analysis	51
3. 3	Results	52
3.3.1	GST as a supraphysiological heat-sensitive client for the chaperone activity of CLU	52
3.3.2	Developing models for the chaperone activity of CLU at physiologically relevant temperatures.	55

3.3.2.1	Investigations of protein thermostability	55
3.3.2.2	The effect of CLU on heat-induced protein precipitation.....	57
3.3.2.3	The effect of temperature of the secondary structure of CLU	64
3.4	Discussion.....	67
4	IDENTIFICATION OF PUTATIVE ENDOGENOUS PLASMA	
	CLIENT PROTEINS FOR CLU.....	70
4.1	Introduction	70
4.2	Materials and methods	71
4.2.1	Protein purification.....	71
4.2.2	Analysis of proteins co-purifying with CLU from untreated human plasma.....	71
4.2.2.1	Western blot	71
4.2.2.2	ELISA	72
4.2.2.3	Flow cytometry	72
4.2.3	Precipitation assays	73
4.2.4	Preparation of residual stressed control proteins.....	73
4.2.5	SEC	73
4.2.6	Plasma treatments	74
4.2.7	Analysis of plasma protein associations with CLU in stressed human plasma.....	74
4.2.7.1	Sandwich ELISA for the identification of stressed client proteins	74
4.2.7.2	SEC to determine the size of putative CLU-FGN complexes in stressed plasma.....	77
4.2.8	Anti-CLU immunoaffinity chromatography for the identification of proteins complexed with CLU.....	77
4.2.8.1	SEC	77
4.2.8.2	SDS-PAGE.....	78
4.2.8.3	Mass spectrometry	78
4.2.8.4	Western blot detection of FGN co-purifying with CLU	78
4.2.8.5	ELISA for the detection of anti-CLU immunoaffinity co-purifying CLU-FGN complexes.....	78
4.3	Results.....	79
4.3.1	Analysis of “contaminant” proteins co-purifying with CLU from unstressed human plasma	79

4.3.2	Purification of CLU-stressed protein complexes formed under oxidative conditions.....	83
4.3.2.1	Lysozyme (LYS) as a model client protein for the chaperone activity of CLU during oxidative stress.....	83
4.3.2.2	<i>In vitro</i> methods for the formation of oxidized CLU-IgG complexes.....	86
4.3.3	Analysis of plasma protein associations with CLU in stressed human plasma.....	88
4.3.4	Analysis of proteins co-purifying with CLU from stressed plasma.....	91
4.3.5	An <i>in vitro</i> method to form and purify HMW CLU-FGN complexes	94
4.3.6	Investigations of the chaperone activity of FGN compared to CLU	99
4. 4	Discussion.....	100
5	CHARACTERIZATION OF HMW CLU-STRESSED PROTEIN COMPLEXES	107
5. 1	Introduction.....	107
5. 2	Materials and methods	108
5.2.1	Purification of HMW CLU-stressed protein complexes.....	108
5.2.2	Preparation of residual stressed protein controls.....	108
5.2.3	Transmission electron microscopy (TEM).....	108
5.2.4	Dynamic light scattering (DLS)	109
5.2.5	Densitometry	109
5.2.6	BisANS fluorescence	110
5.2.7	Thioflavin T fluorescence.....	110
5.2.8	CD analysis.....	110
5. 3	Results.....	111
5.3.1	Quality control analysis of SEC purified HMW CLU-stressed protein complexes.....	111
5.3.2	TEM.....	112
5.3.3	DLS	113
5.3.4	Estimates of stoichiometry	115
5.3.5	Estimates of exposed hydrophobicity.....	116
5.3.6	Thioflavin T analysis.....	118
5.3.7	CD analysis.....	119
5. 4	Discussion.....	123

6 POTENTIAL RECEPTORS FOR HMW CLU-STRESSED PROTEIN COMPLEXES	128
6.1 Introduction	128
6.2 Materials and methods	130
6.2.1 Maleylation of BSA	130
6.2.2 Cell culture	130
6.2.3 Isolation of peripheral human leukocytes	131
6.2.4 Isolation of hepatocytes and non-parenchymal rat liver cells	131
6.2.5 Isolation of rat splenocytes	132
6.2.6 Flow cytometry	132
6.2.6.1 Receptor screening	132
6.2.6.2 Binding assays	133
6.2.6.3 Inhibition of binding to cell surface receptors	134
6.2.7 Surface plasmon resonance	134
6.2.8 Confocal microscopy	135
6.2.9 Protein degradation assays	135
6.2.10 Identification of CLU receptors from plasma membrane protein preparations	136
6.2.10.1 Liver plasma membrane protein isolation	136
6.2.10.2 CLU affinity chromatography of isolated membrane	137
6.3 Results	138
6.3.1.1 Cell binding assays involving uncomplexed CLU	138
6.3.1.1.1 BN cells	138
6.3.1.1.2 HepG2 cells	141
6.3.1.1.3 JEG3 cells	144
6.3.1.1.4 Jurkat cells	145
6.3.1.2 Surface plasmon resonance	145
6.3.2 Binding interactions involving HMW CLU-stressed protein complexes	147
6.3.2.1 Cell binding assays	147
6.3.2.1.1 BN cells	147
6.3.2.1.2 Monocytes	150
6.3.2.1.3 Splenocytes	152
6.3.2.1.4 Isolated rat liver cells	153
6.3.2.2 Confocal microscopy	156

6.3.3	Protein degradation assays.....	159
6.3.4	CLU affinity chromatography.....	160
6.4	Discussion.....	162
7	CLEARANCE OF BLOOD-BORNE HMW CLU-STRESSED PROTEIN COMPLEXES <i>IN VIVO</i>.....	170
7.1	Introduction.....	170
7.2	Materials and methods	172
7.2.1	Purification of HMW CLU-stressed protein complexes.....	172
7.2.2	Iodination.....	172
7.2.3	SPECT imaging.....	173
7.2.4	Preliminary biodistribution.....	174
7.2.5	Biodistribution studies investigating the effect of pre-injection with fucoidin.....	174
7.3	Results.....	176
7.3.1	¹²³ I labelling of CLU, client proteins and HMW CLU-stressed protein complexes.....	176
7.3.2	SPECT imaging.....	177
7.3.3	Preliminary biodistribution study	184
7.3.3.1	Clearance of blood-borne radioactivity after injection with ¹²³ I-labelled HMW CLU-stressed protein complex or uncomplexed control proteins.....	184
7.3.3.2	Organs of highest uptake	186
7.3.4	Biodistribution studies investigating the effect of pre-injection with fucoidin.....	191
7.3.4.1	Clearance of blood-borne radioactivity after injection with ¹²³ I-labelled HMW CLU-stressed protein complexes or control proteins.....	191
7.3.4.2	Organs of highest uptake	194
7.3.4.2.1	Liver.....	198
7.3.4.2.2	Spleen	200
7.3.4.2.3	Kidney	203
7.3.4.3	Other organs.....	205
7.3.4.3.1	Lungs	205
7.3.4.3.2	Thyroid	207
7.3.4.3.3	Stomach	210

7.4	Discussion.....	212
7.4.1	SPECT imaging.....	212
7.4.2	Preliminary biodistribution study	212
7.4.3	Blood-borne clearance of HMW CLU-stressed protein complexes	213
7.4.4	Major organs of uptake	216
7.4.5	Other organs.....	218
7.4.5.1	Uptake of free ¹²³ I and iodinated metabolites	219
7.4.6	Summary.....	219
8	CONCLUSIONS	221
9	REFERENCES	227
	Appendix	252

LIST OF FIGURES

Figure 1.1 Potential fates of unfolding proteins.	5
Figure 1.2 Diagrammatic representation of mechanisms of intracellular protein folding quality control.	12
Figure 1.3 Targeted degradation of non-native proteins via the ubiquitin-proteasome pathway.	14
Figure 1.4 Lysosomal degradation of non-native proteins.....	16
Figure 1.5 Predicted structure of the extracellular chaperone CLU, a disulfide-linked heterodimeric glycoprotein.	23
Figure 1.6 A model for the disposal of stressed extracellular proteins.....	30
Figure 1.7 The structural organization of mammalian LDL superfamily receptors.....	32
Figure 1.8 Schematic representations of the structures of SR classes A-F.	35
Figure 3.1 Inhibition of heat-induced GST precipitation by CLU.....	52
Figure 3.2 SEC of heat stressed or native CLU and GST.	54
Figure 3.3 Detection of HMW CLU-GST complexes by sandwich ELISA.	54
Figure 3.4 Heat-induced precipitation of CPK at 60°C, 50°C and 43°C.	56
Figure 3.5 The effect of CLU on the heat-induced precipitation of CS, LDH, CPK and COL.	58
Figure 3.6 SDS-PAGE of COL and CLU incubated at 43°C in the presence or absence of 10 mM EDTA.	59
Figure 3.7 SEC of heat stressed or native CLU and CS.	60
Figure 3.8 Detection of HMW CLU-CS complexes by sandwich ELISA.	61
Figure 3.9 SEC of heat stressed or native CLU and LDH.	62
Figure 3.10 SEC of heat stressed or native CLU and CPK.	63
Figure 3.11 Native gel of LDH and CLU.....	64
Figure 3.12 CD spectra of CLU during heating between 4-45°C.	65
Figure 3.13 Predicted content of disordered structure for CLU following heating at 4-45°C.....	66
Figure 4.1 Ion-exchange profile and corresponding SDS-PAGE of proteins purified from unstressed human plasma by anti-CLU immunoaffinity chromatography.	80

Figure 4.2 Western blot of "contaminant" protein fraction from ion-exchange chromatography of anti-CLU immunoaffinity proteins purified from human plasma.	81
Figure 4.3 Detection of putative CLU-IgG complexes in the "contaminant" protein fraction (from CLU immunoaffinity chromatography) by sandwich ELISA.....	82
Figure 4.4 The binding of CLU/IgG to isolated leukocytes.....	83
Figure 4.5 Inhibition of oxidative stress-induced LYS precipitation by CLU.	85
Figure 4.6 SEC of native or oxidized CLU and LYS.....	85
Figure 4.7 Detection of CLU-LYS complexes by sandwich ELISA.....	86
Figure 4.8 Inhibition of oxidative stress-induced precipitation of IgG by CLU.	87
Figure 4.9 SEC of native or oxidized CLU and IgG.	87
Figure 4.10 Relative turbidity of plasma stored static at 4°C compared to plasma gently rotated at 37°C for 10 days.....	89
Figure 4.11 Sandwich ELISAs measuring the relative association of major plasma proteins with endogenous plasma CLU after various treatments.....	90
Figure 4.12 SEC of CLU and proteins co-purifying with CLU after incubation of plasma with gentle rotation at 37°C or static storage at 4°C for 10 days..	92
Figure 4.13 SDS-PAGE of protein co-purifying with CLU from human plasma that was for 10 days stored static at 4°C or gently rotated at 37°C.	93
Figure 4.14 Anti-FGN immunoblot of proteins co-purifying with CLU from human plasma that was for 10 days stored static at 4°C or gently rotated at 37°C.....	93
Figure 4.15 Detection of putative CLU-FGN complexes in human plasma by sandwich ELISA.	94
Figure 4.16 Anti-FGN and anti-CLU dot blot analysis of SEC fractionated plasma stored static at 4°C for 10 days or incubated with gentle rotation at 37°C.....	95
Figure 4.17 The effect of macromolecular crowding on precipitation of FGN.	96
Figure 4.18 Inhibition of heat-induced precipitation of FGN by CLU.....	97
Figure 4.19 SEC of heat stressed or native CLU and FGN.....	98
Figure 4.20 Detection of CLU-FGN complexes by sandwich ELISA.	98
Figure 4.21 Inhibition of heat-induced CS precipitation by CLU or FGN.	99

Figure 5.1 SEC analysis of HMW CLU-stressed protein complexes stored at 4°C for 3 months.	111
Figure 5.2 TEM images of native and stressed LYS, GST and CS in the presence of CLU.	113
Figure 5.3 DLS estimates of the mean diameters of HMW CLU-stressed protein complexes and uncomplexed native and residual heated control proteins.	114
Figure 5.4 Image of a reducing SDS-PAGE gel of HMW CLU-GST.	115
Figure 5.5 Plots showing concentration dependence of bisANS fluorescence for HMW CLU-stressed protein complexes and native or heated client proteins.	117
Figure 5.6 Thioflavin T fluorescence of HMW CLU-GST, HMW CLU-FGN and relevant native and heated control proteins compared to a sample of LYS amyloid.	119
Figure 5.7 Near UV CD spectra for HMW CLU-client protein complexes and other protein samples.	121
Figure 5.8 CDSSTR predictions from near UV CD data of HMW CLU-stressed protein complexes and other protein samples.	122
Figure 6.1 Surface expression of megalin by BN cells, assessed by flow cytometry. .	138
Figure 6.2 The effect of pre-incubation with GST-RAP on the binding of CLU and RAP to BN cells, assessed by flow cytometry.	139
Figure 6.3 Dose-dependant binding of CLU to BN cells, assessed by flow cytometry.	140
Figure 6.4 The effect of EDTA or pre-treatment with trypsin on the binding of CLU and RAP to BN cells, assessed by flow cytometry.	141
Figure 6.5 Surface expression of LRP by HepG2 cells, assessed by flow cytometry.	142
Figure 6.6 Dose-dependant binding of CLU to HepG2 cells, assessed by flow cytometry.	142
Figure 6.7 The effect of pre-incubation with ASF or RAP on the binding of biotinylated CLU to HepG2 cells, assessed by flow cytometry.	143
Figure 6.8 The effect of excess unlabelled CLU on the binding of biotinylated CLU to HepG2 cells, assessed by flow cytometry.	143
Figure 6.9 Surface expression of LRP by JEG3 cells, assessed by flow cytometry....	144

Figure 6.10 The effect of pre-incubation with RAP or an inhibitory anti-LRP antibody on the binding of biotinylated CLU to JEG3 cells, assessed by flow cytometry.	144
Figure 6.11 The effect of pre-incubation with galactose on the binding of CLU to Jurkat cells, assessed by flow cytometry.	145
Figure 6.12 Surface plasmon resonance measurements of binding to megalin and LRP.....	146
Figure 6.13 The effect of pre-incubation with GST-RAP on the binding of biotinylated CLU, FGN and HMW CLU-FGN to BN cells, assessed by flow cytometry.	148
Figure 6.14 The effect of fucoidin on the binding of biotinylated HMW CLU-stressed protein complexes and control proteins to BN cells, assessed by flow cytometry.....	149
Figure 6.15 The effect of mBSA on the binding of biotinylated HMW CLU-FGN and CLU to BN cells, assessed by flow cytometry.	150
Figure 6.16 Binding of HMW CLU-stressed protein complexes, CLU and uncomplexed client proteins to isolated human leukocytes, assessed by flow cytometry.	151
Figure 6.17 The effect of fucoidin on the binding of HMW CLU-stressed protein complexes and control proteins to peripheral blood CD14+ monocytes, assessed by flow cytometry.....	152
Figure 6.18 The effect of fucoidin on the binding of biotinylated HMW CLU-stressed protein complexes and control proteins to rat splenocytes, assessed by flow cytometry.....	153
Figure 6.19 The binding of biotinylated HMW CLU-stressed protein complexes and control proteins to isolated rat liver cells, assessed by flow cytometry...	154
Figure 6.20 The binding of HMW CLU-stressed protein complexes and control proteins to isolated rat hepatocytes, assessed by flow cytometry.	155
Figure 6.21 The effect of fucoidin on the binding of HMW CLU-stressed protein complexes and control proteins to rat hepatocytes, assessed by flow cytometry.	156
Figure 6.22 Confocal microscopy images of ALEXA488 labelled HMW CLU-FGN bound to the surface of a BN cell at 4°C.	157

Figure 6.23 Confocal microscopy images of BN cells after a 1 h incubation at 37°C with 100 µg/mL ALEXA488 labelled HMW CLU-FGN and 200 nM Lysotracker Red DND-99.	157
Figure 6.24 Confocal images of ALEXA488 labelled HMW CLU-FGN bound to the surface of rat hepatocytes.	158
Figure 6.25 Confocal images of internalized ALEXA488 labelled HMW CLU-FGN and Lysotracker Red DND-99 fluorescence in a rat hepatocyte.....	159
Figure 6.26 Western blot of cell lysates of rat hepatocytes incubated for 1-3 h at 37°C with 250 µg/mL biotinylated HMW CLU-GST in the presence or absence of 100 µM chloroquine.....	160
Figure 6.27 12% SDS-PAGE of bovine liver membrane proteins isolated by CLU affinity chromatography.	161
Figure 7.1 SEC of ¹²³ I-labelled CLU and reaction by-products.....	177
Figure 7.2 SEC of purified ¹²³ I-labelled CLU.....	177
Figure 7.3 SPECT imaging of a Sprague Dawley rat injected with ¹²³ I-HMW CLU-FGN.	179
Figure 7.4 SPECT imaging of a Sprague Dawley rat injected with ¹²³ I-FGN.....	180
Figure 7.5 SPECT imaging of a Sprague Dawley rat injected with ¹²³ I-CLU.	181
Figure 7.6 SPECT imaging of a Sprague Dawley rat injected with ¹²³ I-HMW CLU-GST.	182
Figure 7.7 SPECT imaging of a Sprague Dawley rat injected with ¹²³ I- GST.....	183
Figure 7.8 Percentage of the injected dose/g blood in Sprague Dawley rats after injection with ¹²³ I-labelled HMW CLU-stressed protein complexes or uncomplexed control proteins.	185
Figure 7.9 Total percentage of radioactivity remaining in the blood of Sprague Dawley rats 1 h after injection of ¹²³ I-labelled HMW CLU-stressed protein complexes or uncomplexed control proteins.	186
Figure 7.10 Percentage of the injected dose/g of tissue in Sprague Dawley rats 1 h after injection of ¹²³ I-labelled HMW CLU-stressed protein complexes or uncomplexed control proteins.	187
Figure 7.11 Percentage of the injected dose/g tissue in Sprague Dawley rats 1, 6 and 24 h after injection with ¹²³ I-labelled HMW CLU-GST, *CLU or *GST.....	189
Figure 7.12 Percentage of the injected dose/g tissue in Sprague Dawley rats 1, 6 and 24 h after injection of ¹²³ I-labelled HMW CLU-FGN, *CLU or *FGN.....	190

Figure 7.13 Percentage of the injected dose/g blood in Sprague Dawley rats after injection with ^{123}I -labelled HMW CLU-stressed protein complexes or uncomplexed control proteins.	192
Figure 7.14 Percentage of the injected dose remaining in the blood of Sprague Dawley rats 1 h after injection of ^{123}I -labelled HMW CLU-stressed protein complexes or uncomplexed control proteins.	193
Figure 7.15 Ratios of the proportion of injected dose in the blood of fucoidin pre-treated versus control Sprague Dawley rats injected with ^{123}I -labelled HMW CLU-stressed protein complexes or uncomplexed control proteins at (A) 5 min, and (B) 15 min p.i.	194
Figure 7.16 Percentage of the injected dose/g of tissue in Sprague Dawley rats 5 min after injection with ^{123}I -labelled HMW CLU-CS, CLU or CS without (A) or with (B) fucoidin pre-treatment.	196
Figure 7.17 Percentage of the injected dose/g of tissue in Sprague Dawley rats 5 min after injection with ^{123}I -labelled HMW CLU-FGN, CLU or FGN without (A) or with (B) fucoidin pre-treatment.	197
Figure 7.18 Percentage of the injected dose/g liver in Sprague Dawley rats after injection with ^{123}I -labelled HMW CLU-stressed protein complexes or uncomplexed control proteins.	199
Figure 7.19 Ratios of the proportion of liver-associated injected dose for fucoidin pre-treated versus control Sprague Dawley rats injected with ^{123}I -labelled HMW CLU-stressed protein complexes or uncomplexed control proteins at (A) 5 min, and (B) 15 min p.i.	200
Figure 7.20 Percentage of the injected dose/g spleen in Sprague Dawley rats after injection with ^{123}I -labelled HMW CLU-stressed protein complexes or uncomplexed control proteins.	201
Figure 7.21 Ratios of the proportion of spleen-associated injected dose for fucoidin pre-treated versus control Sprague Dawley rats injected with ^{123}I -labelled HMW CLU-stressed protein complexes or uncomplexed control proteins at (A) 5 min, and (B) 15 min p.i.	202
Figure 7.22 Percentage of the injected dose/g kidney in Sprague Dawley rats after injection with ^{123}I -labelled HMW CLU-stressed protein complexes or uncomplexed control proteins.	203

Figure 7.23 Ratios of the proportion of kidney-associated injected dose for fucoidin pre-treated versus control Sprague Dawley rats injected with ¹²³ I-labelled HMW CLU-stressed protein complexes or uncomplexed control proteins at (A) 5 min, and (B) 15 min p.i.	204
Figure 7.24 Percentage of the injected dose/g lung in Sprague Dawley rats after injection with ¹²³ I-labelled HMW CLU-stressed protein complexes or uncomplexed control proteins.	206
Figure 7.25 Ratios of the proportion of lung-associated injected dose for fucoidin pre-treated versus control Sprague Dawley rats injected with ¹²³ I-labelled HMW CLU-stressed protein complexes or uncomplexed control proteins at (A) 5 min, and (B) 15 min p.i.	207
Figure 7.26 Percentage of the injected dose/g thyroid in Sprague Dawley rats after injection with ¹²³ I-labelled HMW CLU-stressed protein complexes or uncomplexed control proteins.	208
Figure 7.27 Ratios of the proportion of thyroid-associated injected dose for fucoidin pre-treated versus control Sprague Dawley rats injected with ¹²³ I-labelled HMW CLU-stressed protein complexes or uncomplexed control proteins at (A) 60 min, and (B) 30 min p.i.....	209
Figure 7.28 Percentage of the injected dose/g stomach in Sprague Dawley rats after injection with ¹²³ I-labelled HMW CLU-stressed protein complexes or uncomplexed control proteins.	210
Figure 7.29 Ratios of the proportion of stomach-associated injected dose for fucoidin pre-treated versus control Sprague Dawley rats injected with ¹²³ I-labelled HMW CLU-stressed protein complexes or uncomplexed control proteins at (A) 60 min, and (B) 30 min p.i.	211

LIST OF TABLES

Table 1.1	Examples of conditions causing elevated temperatures in humans.....	4
Table 1.2	Examples of PDDs and the proteins implicated in their pathology.	6
Table 1.3	Receptors for normally intracellular chaperones.	20
Table 1.4	Overexpression of CLU in disease.	25
Table 1.5	Classification and ligands of SRs.	34
Table 2.1	Antibodies used in this study and their respective suppliers.....	40
Table 3.1	Conditions for the preparation of residual heated controls.	49
Table 3.2	Minimum temperatures required to induce the precipitation of COL, LDH, CPK and CS within a 24 h period.....	56
Table 4.1	Details of sandwich ELISAs used to detect the stress-induced association of CLU with 11 major plasma proteins.	76
Table 5.1	Approximate ratio of CLU to stressed client protein in SEC purified HMW CLU-FGN, HMW CLU-CS and HMW CLU-GST complexes.....	116
Table 5.2	Binding parameters for the binding of bisANS to HMW CLU-stressed protein complexes and heated or native client proteins.....	118
Table 6.1	Kinetic data for the binding of CLU and HMW CLU-stressed protein complexes to megalin.	147

ABBREVIATIONS

°C	degrees celsius
41D	monoclonal anti-clusterin antibody
Å	angstrom
α_2 M	α_2 -macroglobulin
A280	absorbance at 280 nm
A360	absorbance at 360 nm
A490	absorbance at 490 nm
ACID GLY	α_1 acid glycoprotein
AcLDL	acetylated low density lipoprotein
AFU	arbitrary fluorescence units
AGE	advanced glycation end product
AIF	apoptosis inducing factor
ALEXA488	Alexa fluor [®] 488
ALEXA633	Alexa fluor [®] 633
AMD	age-related macular degeneration
ApoAI	apolipoprotein AI
ApoE	apolipoprotein E
ApoER2	apolipoprotein E receptor 2
ASF	asialofetuin
ATP	adenosine triphosphate
Az	sodium azide
A β	amyloid-beta
BD	Becton, Dickinson and Company
bisANS	4,4'-Bis(1-anilino-8-naphthalene sulfonate)
BSA	bovine serum albumin
CD	circular dichroism
CLU	clusterin
COL	collagenase IV
CPK	creatine phosphokinase
CS	citrate synthase
CSF	cerebral spinal fluid
Da	daltons
DCM	dichloromethane
DLS	dynamic light scattering
DMEM:F-12	Dulbecco's modified Eagles medium: Hams F-12
DMSO	deoxymethylsulfoxide
DNA	deoxyribonucleic acid
dSR-CI	drosophila scavenger receptor class C type I
DTT	dithiothreitol

<i>E. coli</i>	<i>Escherichia coli</i>
ECL	enhanced chemiluminescence
EDTA	ethylene diamine tetraacetic acid
EGF	epidermal growth factor
EGTA	ethylene glycol tetraacetic acid
ELISA	enzyme linked immunosorbent assay
Em	emission
ER	endoplasmic reticulum
ERAD	endoplasmic reticulum-associated protein degradation
Ex	excitation
F	F statistic
FCS	fetal calf serum
FEEL-1	fasciclin EFG-like, laminin-type EFG-like, and link domain-containing scavenger receptor type 1
FEEL-2	fasciclin EFG-like, laminin-type EFG-like, and link domain-containing scavenger receptor type 2
FGN	fibrinogen
FITC	fluorescein isothiocyanate
FPLC	fast protein liquid chromatography
<i>g</i>	relative centrifugal force (9.8 m.s ⁻²)
G7	monoclonal anti-CLU antibody
GST	glutathione-S-transferase
GST-RAP	glutathione-S-transferase-receptor-associated protein (fusion protein)
h	hour(s)
HBB	Hank's binding buffer
HDC	heat denatured casein
HDC/PBS	1% heat denatured casein and 0.01% thimerosal in phosphate buffered saline.
HDL	high density lipoprotein
HMW	high molecular weight
Hp	haptoglobin
HPLC	high performance liquid chromatography
HRP	horseradish peroxidase
HSA	human serum albumin
Hsc	heat-shock cognate protein
HSD	honestly significant difference
Hsp	heat-shock protein
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
IL	interleukin

IPTG	isopropyl- β -D-thiogalactopyranoside
K _d	dissociation constant
kDa	kilo daltons
Lamp 2a	lysosome-associated membrane protein type 2a
LB	Luria-Bertani
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LDLR	low density lipoprotein receptor
LOX-1	lectin-like oxidized low density lipoprotein receptor
LRP	low density lipoprotein receptor-related protein
ly-hsc73	lysosomal hsc73
LYS	lysozyme (hen egg)
MAC	membrane attack complex
MARCO	macrophage receptor with collagenous structure
mBSA	maleylated bovine serum albumin
mg	milligrams
min	minutes
mL	millilitres
mm	millimetres
mM	millimolar
Mr	marker
MW	molecular weight
m/z	mass to charge
μ g	micrograms
μ L	microlitres
μ m	micrometres
μ M	micromolar
n	number
NAMIDD	non-amyloidotic monoclonal IgG deposition disease
ng	nanograms
nm	nanometres
nM	nanomolar
NRD	no reported data
OPD	<i>o</i> -phenylenediamine dihydrochloride
OVA	ovalbumin
oxLDL	oxidized low density lipoprotein
PARP	poly ADP ribose polymerase
p	statistical significance
p.i.	post-injection
PBS	phosphate buffered saline
PDDs	protein deposition diseases

PEG	polyethylene glycol
pH	power of hydrogen
PI	propidium iodide
RAGE	receptor for advanced glycation end products
RAP	receptor-associated protein
ROS	reactive oxygen species
rpm	revolutions per min
s	seconds
SA	streptavidin
SAP	serum amyloid P component
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SEC	size exclusion chromatography
sHsps	small heat-shock proteins
SOD	superoxide dismutase
SPECT	single photon emission computed tomography
SR-AI	scavenger receptor class A type I
SR-AII	scavenger receptor class A type II
SR-AIII	scavenger receptor class A type III
SR-BI	scavenger receptor class B type I
SRCR	scavenger receptor cysteine-rich domain
SREC-1	scavenger receptor expressed by endothelial cells
SR-PSOX	scavenger receptor for phosphatidylserine and oxidized low density lipoprotein
TAE	TRIS acetate EDTA
TEM	transmission electron microscopy
TMED	<i>N,N,N',N'</i> -tetramethylethylenediamine
TRANS	transferrin
TRIS	2-amino-2-hydroxymethyl-propane-1,3-diol
tRNA	transfer ribonucleic acid (not defined in text)
TRYP	α_1 antitrypsin
UPR	unfolded protein response
UV	ultraviolet
V	volts
v/v	volume per volume
VLDL	very low density lipoprotein
VLDLR	low density lipoprotein receptor
V _o	void volume, exclusion limit
w/v	weight per volume
Z	Z score

PUBLICATIONS AND CONFERENCE PRESENTATIONS

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Yerbury, J. J., Stewart, E. M., Wyatt, A. R. and M. R. Wilson (2005) Quality control of protein folding in extracellular space. *EMBO Reports* 6: 1131-1136

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Conference Presentations

2006 International Biotechnology and Medical Science Student Forum, Beijing, China.
Oral presentation titled: The role of clusterin in extracellular protein quality control.

2007 World Conference of Stress, Budapest, Hungary.
Poster presentation titled: Its time to take out the trash! – Chaperone-dependent disposal of unfolded proteins via LDL superfamily receptors.

2007 International Society of Neurochemistry, Protein Misfolding and Neurodegenerative Disease Meeting, Dunk Island, Australia.
Oral presentation titled: The role of clusterin in extracellular protein quality control.

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- 2008 5th Clusterin/ApoJ Workshop, Spetes, Greece
Oral presentation titled: The role of clusterin in extracellular protein quality control.
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Oral presentation titled: The role of clusterin in extracellular protein quality control.